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TITLE: Temporal Patterns of Mammary Epithelial Cell Gene Expression in Response to Glucocorticoid Receptor Activation

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13. Abstract *(Maximum 200 Words) (abstract should contain no proprietary or confidential information)*

Because GR-mediated transcriptional regulation is required for the potent survival signal observed in MECs we hypothesized that the identification of key targets of GR-activation may lead to novel targets for breast cancer therapy. One approach to hone in on physiologically relevant genes is to analyze multiple time points for gene induction and repression by dexamethasone using microchip technology. This approach successfully allowed us to monitor gene expression at successive times in cells undergoing apoptosis in response to serum withdrawal and compare this set of genes to those expressed over time in cells protected from apoptosis by GR activation. The **concept** to be tested was that we might efficiently identify relevant pathways involved in this novel survival signaling pathway by using cluster analysis to examine temporal patterns of expression rather than by simply cataloguing individual genes induced in an array at a single time point following GR activation. We achieved this goal using Affymetrix chips and monitoring gene expression over or under baseline 30 minutes, 2 hoursm, 4 hours and 24 hours following GR activation. Several of the gene products we identified are players in key signal tranduction pathways involved in cell survival.

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SUMMARY OF PROPOSAL RESULTS:

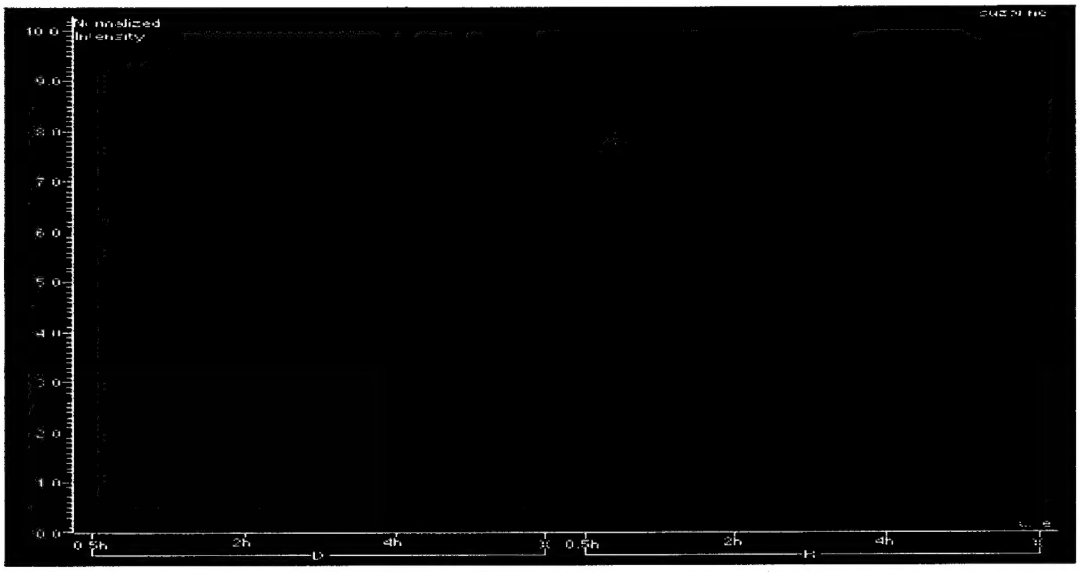
We and others have previously demonstrated that glucocorticoid receptor (GR) activation in mammary epithelial cells (MECs) initiates a survival signal. To identify the mechanisms involved in GR-mediated cell survival of MECs, we studied GR-induced gene expression by hybridizing transcripts from glucocorticoid-treated MECs to high-density oligonucleotide arrays representing over 12,000 human genes.

An average of 11,127 transcripts (>80%) was detected in three independent experiments using RNA derived from MECs treated for 30 minutes with either: 1) vehicle (ethanol) alone, 2) dexamethasone (10-6M) or 3) a combination of dexamethasone (10-6M) and the GR antagonist RU486 (10-7M). Each experiment was repeated independently on three different occasions and all data were then compared and analyzed using Genechip Analysis Software Suite 4.0 (Affymetrix) and GeneSpringTM software.

Ninety-five GR-induced genes were identified as being consistently expressed at least 1.5-fold over control (vehicle alone) transcripts in all three experiments. Thirty-four of the 95 induced genes were also consisitently repressed following concomitant dexamethasone and RU486 treatment. In addition, 69 genes were found to be down-regulated at least 0.5-fold following dexamethasone treatment. The GR-responsive genes appear to cluster into either signal transduction, cell cycle and apoptosis, metabolism, transcription, protein synthesis/processing, or growth receptor-related functional groups. Preliminary data reveal that the first four genes examined by Northern blot are reprodicubly upregulated by glucocorticoid and inhibited by concomittant RU486. Additional studies examining the expression and potential survival functions of these genes and their encoded proteins are ongoing. Duplicate time course experiments examining gene expression at four time points from 30 minutes to 24 hours following GR activation was also performed using the funding from this Concept Award. Temporal patterns of gene expression revealed consistent patterns of signal transduction pathway modulated by GR activation. Interestingly, the most prominent peak in gene expression was at two hours and the majority of these genes are directy involved in signal transduction pathways.

In summary, we have successfuly identified patterns of gene expression using the genome wide array expression techniques. As hypothesized in the original Concept Award application, this information has allowed us to link GR signaling to pathways not previously connected to glucocorticoid action. This Concept Award funding enabled my laboratory to do the preliminary gene array experiments needed as a foundation for ongoing experiments examining the mechanisms by which GR activation can modulate signal transduction pathways.

Figure 1: Overview of genes modulated by dex or dex/RU486



30min 2h 4h 24h 30min 2h 4h 24h
DEXAMETHASONE DEXAMETHASONE/RU486

KEY RESEARCH ACCOMPLISHMENTS

Identification of several genes regulated by GR that have never been linked to GR signaling previously.

REPORTABLE OUTCOMES

We are preparing a manuscript describing the pattern of gene activation.

CONCLUSIONS

GR activation in mammary epithelial cells activates and represses a number of genes involved in survival signal transduction pathways. We have conclusively linked GR activation to the PI3-kinase-SGK survival signaling pathway.

REFERENCES

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APPENDICES

None.